# ISOLATION OF POLYMERIZATION-COMPETENT VIMENTIN FROM PORCINE EYE LENS TISSUE

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## 1. Introduction

Most higher cells contain '10 nm filaments' (intermediate filaments) as third cytoskeletal component in addition to microtubules and actin-containing microfilaments. Immunological and gel electrophoretic studies have distinguished several distinct intermediate filament subclasses, each related to certain cell and tissue types (review [1,2]). Whereas neurofilaments, glial filaments, muscle desmin filaments and epidermal tonofilaments are readily available from corresponding tissues, it has consistently remained difficult to obtain vimentin in larger amounts. Vimentin is found in mesenchymal cells and also expressed in many, if not all, permanent cell lines [1,4]. Denatured vimentin has been purified in small amounts by extraction of cytoskeletons of cultured cells using preparative gel electrophoresis [1–4]. In addition, native intermediate filaments have been isolated in bundled form from cultured BHK 21 cells [5], but subsequent studies revealed that they contain not only vimentin, but also desmin [6,7] and some higher molecular weight (or $M_r$) proteins [8].

In order to explore the molecular basis of different intermediate filaments, protein–chemical characterization is required and in the case of vimentin an easy purification scheme is demanded. Here we report that eye lens tissue, which is known to contain vimentin by immunological criteria [9] is an excellent source to obtain purified vimentin in good yield. The isolated protein renatures spontaneously into filaments after urea treatment. The purification scheme described eliminates the need for costly tissue culture facilities and provides amounts suitable for further biochemical and protein–chemical characterizations.

## 2. Materials and methods

Pig eyes from the slaughterhouse were immediately dissected. Lenses (43 g) were homogenized in the cold for 1 min in 350 ml 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$ and 10 mM 2-mercaptoethanol using a Polytron device (Kinematica GmbH, Kriens). Centrifugation at 17 000 rev./min for 30 min provided a pellet, which was washed twice with the same buffer. The final pellet was extracted at 4°C with 100 ml 8 M urea in 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 5 mM DTT for 4 h. After removal of insoluble residue by centrifugation for 30 min at 76 000 × *g* an equal volume of buffer without urea was added to the supernatant. Centrifugation at 76 000 × *g* for 45 min provided a supernatant from which proteins were collected by addition of 6 vol. cold ethanol and low speed centrifugation. The pellet was dissolved in 20 ml 7 M urea in 30 mM sodium formate (pH 4.0), 1 mM EGTA, 1 mM DTT. After filtration through Sephadex G-25 (250 ml bed vol.) in the same buffer, the solution was applied at 4°C on a 50 ml carboxymethyl cellulose column (Whatman CM 32, USA) in the same buffer. Vimentin and other proteins were eluted by 400 ml of a linear NaCl gradient (0–0.13 M). Fractions were screened by sodium dodecylsulfate (SDS) – polyacrylamide gel electrophoresis. Those containing vimentin were pooled and protein was recovered by ethanol precipitation. Final purification was achieved by chromatography on DEAE-cellulose (Whatman DE 52). The pellet was dissolved in 4 ml 7 M urea in 50 mM Tris–HCl (pH 7.5), 1 mM EGTA, 1 mM DTT, 10 mM NaCl, filtered through a 50 ml Sephadex G-25 column and applied on DE 52 (10 ml bedsize in the same buffer). Vimentin was eluted by 300 ml of a linear NaCl gradient (10–50 mM NaCl). Fractions were

screened by gel electrophoresis and those containing vimentin of sufficient purity were pooled and the protein was recovered by ethanol precipitation. Vimentin used for protein–chemical studies was suspended in and extensively dialyzed against water at 4°C before being recovered by lyophilization. All other procedures were as described for desmin [10].

## 3. Results

Repeated washing of the crude eye lens tissue homogenate results in a pellet, which is a rich source of vimentin. Extraction with 8 M urea solubilizes most of the vimentin. The subsequent centrifugation step in 4 M urea leaves most of the vimentin soluble, although some protein is lost with the membrane fraction in the pellet. CM 32 chromatography of the urea-soluble proteins (fig.1 A) does not provide a strong enrichment of vimentin but reproducibly removes a contaminating polypeptide of slightly lower $M_r$ than vimentin. Final purification of vimentin is achieved by chromatography on DE 52 cellulose where contaminating low $M_r$ proteins are removed (fig.1B). From 10–20 mg vimentin is obtained from 100 eye lenses.

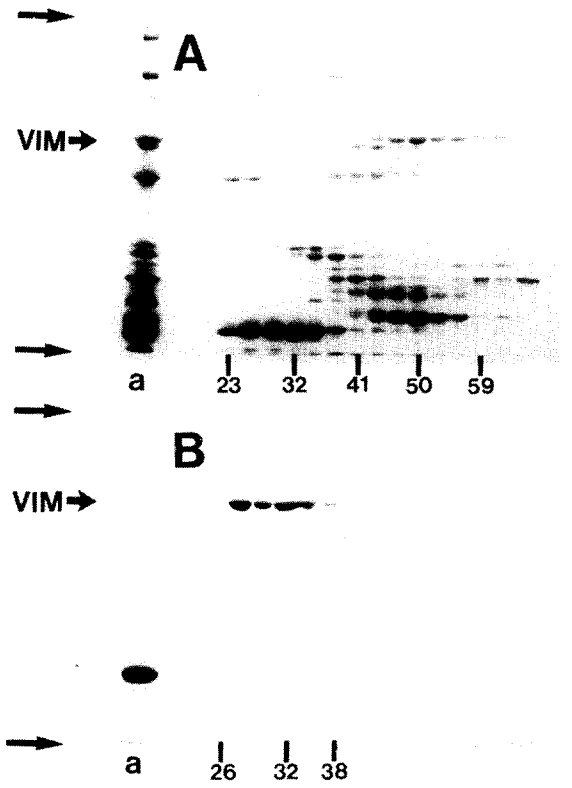


Fig.1. SDS–polyacrylamide gel analysis of proteins eluted from: CM-cellulose column (A); and DEAE-cellulose column (B) (see section 2). In (A) material applied on column is shown in slot (a). Numbers under slots correspond to numbers of appropriate fractions. Note enrichment of vimentin around fraction 50. In (B) proteins eluted in the void volume are shown in slot (a). Numbers under the slots correspond to fractions eluted by salt gradient. Note elution of pure vimentin in fractions 26–35. VIM indicates vimentin.

Purified vimentin dissolved in the 7 M urea solution used for DE 52 chromatography was dialyzed against 50 mM Tris–HCl buffer containing 1 mM DTT and 1 mM $MgCl_2$. Electron microscopy using negative stain analysis revealed extensive filament formation characterized by a diameter of 7–11 nm (fig.2).

Purified vimentin (fig.3) was characterized by two-dimensional gel electrophoresis according to [11]. Vimentin appeared in isoelectric variants of 2–3 spots with the same $M_r$. In addition we also, as expected, found that pig eye lens vimentin and chicken desmin differ in app. $M_r$ and isoelectric point and note the presence of different isoelectric species (see [1,7,9].

Pig eye lens vimentin shows a similar but not identical amino acid composition (table 1) when compared with chicken gizzard and pig stomach desmin [10].

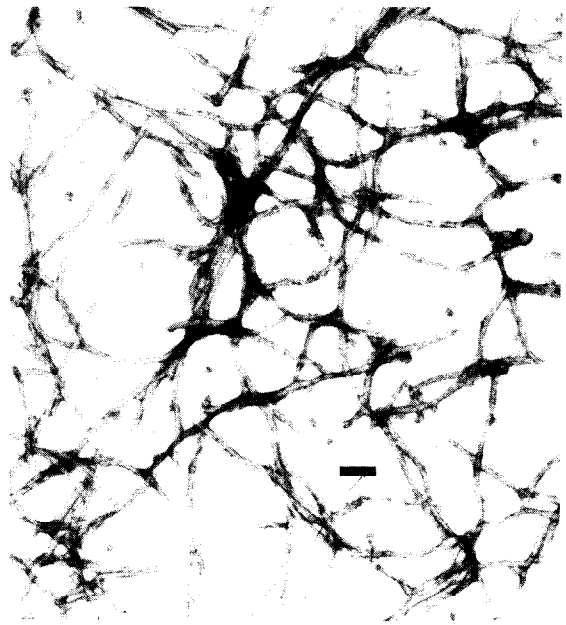

Fig.2. Reconstituted vimentin filaments obtained by dialysis of urea-denatured vimentin against 50 mM Tris–HCl (pH 7.5), 1 mM DTT and 1 mM $MgCl_2$. Bar represents 0.1 μm.

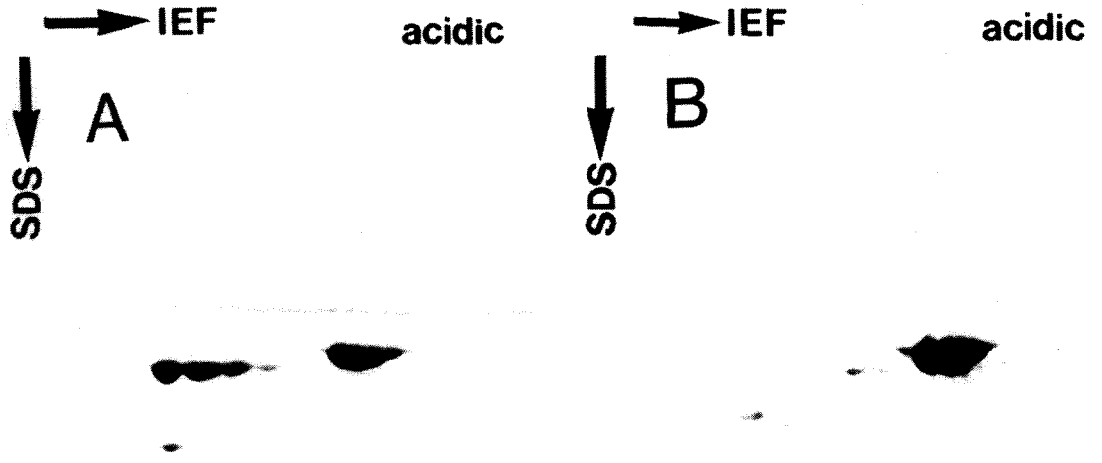


Fig.3. Two-dimensional gelelectrophoresis [11]. The same region of the gels is given in (A) and (B). (A) is pig eye vimentin + chicken gizzard desmin, (B) is pig eye vimentin. IEF means isoelectric focusing and SDS means SDS gel electrophoresis. For desmin alone see [10]. Note the separation of desmin and vimentin in both directions.

Glutamic acid, alanine, leucine and arginine residues are abundantly present in agreement with the highly α-helical structure of proteins of the k–m–e–f group (see, e.g., [12]). End-group analysis reveals a glutamic acid residue both in carboxypeptidase Y digestion (0.3 mol/mol) and hydrazinolysis (0.4 mol/mol). No free amino-terminal end-group was obtained.

Table 1
Amino acid composition of pig eye lens vimentin and chicken gizzard desmin

| | Chicken gizzard desmin | Pig eye lens vimentin |
|---|---|---|
| Asx | 9.5 | 12.8 |
| Thr | 5.7 | 6.2 |
| Ser | 6.6 | 8.9 |
| Glx | 17.8 | 18.5 |
| Pro | 3.0 | 2.7 |
| Gly | 6.1 | 2.7 |
| Ala | 10.4 | 6.3 |
| Cys | 0.2 | 0.2 |
| Val | 5.8 | 5.7 |
| Met | 1.7 | 2.0 |
| Ile | 4.0 | 3.1 |
| Leu | 8.9 | 10.7 |
| Tyr | 2.8 | 2.5 |
| Phe | 2.9 | 2.2 |
| Lys | 4.8 | 4.8 |
| His | 1.7 | 1.4 |
| Arg | 8.7 | 9.2 |

Results are expressed per 100 residues and are based on 24 h of normal hydrolysis. Cysteine was determined after performic acid oxidation. The results for desmin are from [10]

## 4. Discussion

The eye lens is an obvious choice as starting material for the purification of vimentin:

(i) Most cells have a very high ratio of cytoplasm to nucleus because of their typical morphology;
(ii) Intermediate filaments are present in abundant amounts as judged by previous electron microscopical data [9];
(iii) The tissue is readily available from the local slaughterhouse, thus dispensing for the need for large scale tissue culture facilities [5] with their inherent financial burden.

Purified porcine eye lens vimentin shows the same polypeptide app. $M_r$ and isoelectric focusing behavior as vimentin in cytoskeletal preparations [3,6,7,9]. Eye lens vimentin shows a simpler polypeptide composition than preparations of intermediate filaments from BHK 21 cells which revealed in addition to vimentin some desmin and several yet unidentified polypeptides [5–8]. Eye lens vimentin, although homogeneous by one-dimensional gels, is polymerization competent giving rise to morphologically normal intermediate

filaments. Thus the function, if any, of other protein components found in isolated BHK 21 100 Å filaments [5–8] remains unknown.

As expected, a comparison of vimentin and desmin [10] reveals similarities as well as differences. Amino acid analysis shows a rather similar but not identical composition. Similarities in amino acid composition have also been reported for other 100 Å filament proteins [5]. Vimentin and desmin even from the same species (pig) differ in their polypeptide app. $M_r$ values and isoelectric points [1,6,7]. Both have a blocked amino-terminus but differ at their carboxy-terminus. Pig eye lens vimentin and pig stomach desmin [10] prepared by similar procedures show reconstitution into filaments with a morphology closely related to that typically found for intermediate filaments.

Previous results obtained by one- as well as two-dimensional gels and various immunological experiments emphasize the concept of several distinct classes of 10 nm filaments [1,2]. These subgroups can differ markedly as shown by the complexity of the keratin polypeptides (see, e.g., [13]) and the extremely high $M_r$ values obtained for some of the neurofilament polypeptides (for references see [1]). However, the rather similar ultrastructural morphology together with certain biochemical properties indicate the possibility of a common structural principle, which should be recognizable at the amino acid sequence level. To this end, we have started such a study on smooth muscle desmin [10]. The vimentin available by the procedure described, should allow us to extend this study to an immunologically distinct intermediate filament protein.

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**References**

[1] Lazarides, E. (1980) Nature 283, 249–256.
[2] Weber, K. and Osborn, M. (1981) Cell Surface Rev. 7, in press.
[3] Franke, W. W., Schmid, E., Osborn, M. and Weber, K. (1978) Proc. Natl. Acad. Sci. USA 75, 5034–5038.
[4] Franke, W. W., Schmid, E., Winter, S., Osborn, M. and Weber, K. (1979) Exp. Cell Res. 123, 25–46.
[5] Starger, J. M., Brown, W. E., Goldman, A. E. and Goldman, R. D. (1978) J. Cell Biol. 78, 93–109.
[6] Tuszynski, G. P., Frank, E., Damsky, C., Buck, C. A. and Warren, L. (1979) J. Biol. Chem. 254, 6138–6143.
[7] Gard, D. L., Bell, P. B. and Lazarides, E. (1979) Proc. Natl. Acad. Sci. USA 76, 3896–3899.
[8] Zieve, G. W., Heidemann, S. R. and McIntosh, J. R. (1980) J. Cell Biol. 87, 160–169.
[9] Ramaekers, F. C. S., Osborn, M., Schmid, E., Weber, K., Bloemendahl, H. and Franke, W. W. (1980) Exp. Cell Res. 127, 309–327.
[10] Geisler, N. and Weber, K. (1980) Eur. J. Biochem. 111, 425–433.
[11] O'Farrell, P. H. (1975) J. Biol. Chem. 250, 4007–4021.
[12] Steinert, P. M., Idler, W. W. and Goldman, R. D. (1980) Proc. Natl. Acad. Sci. USA 77, 4534–4538.
[13] Steinert, P. M., Idler, W. W. and Wantz, M. L. (1980) Biochem. J. 187, 913–916.